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Intracellular blockade of GABAA receptors in the rat hippocampal neurons

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Abstract

The intracellular blockade of GABAA-receptor-mediated currents is a useful approach to suppress the GABAergic conductance in a single cell and to isolate the glutamatergic component of network-driven activities. Previously an approach has been described allowing intracellular blockade of GABA A receptors by means of intracellular dialysis of a neuron with the pipette-filling solution, in which fluoride ions that hardly pass through the GABAA receptor channels substitute for Cl- and in which Mg2+ and ATP are omitted to induce rundown of the GABAA receptors during whole-cell patch-clamp recordings. However, the kinetics of suppression of GABAergic conductance and the effect on the currents mediated by glutamate receptors remain unknown. Here, using whole-cell recordings with fluoride-based, Mg2+- and ATP-free solution on CA3 hippocampal neurons of neonatal rats, we show that after 1 h of such dialysis, both spontaneous and evoked GABAA-receptor-mediated synaptic currents and responses induced by the GABAA receptor agonist isoguvacine were completely suppressed. Inward GABAergic postsynaptic currents were suppressed prior to outward currents. Synaptic responses mediated by AMPA receptors were not affected by the dialysis, whereas the NMDA-receptor-mediated postsynaptic currents were reduced by approximately 20%. Dialysis with fluoride-based Mg 2+, ATP-free solution either fully blocked giant depolarizing potentials (GDPs) in CA3 pyramidal cells (n = 2) or reduced the charge crossing the membrane during GDPs and shifted the GDP reversal potential to more positive values (n = 5). The dialysis-resistant component of GDPs was mediated by glutamate receptors, since: (i) it reversed around 0 mV; (ii) it demonstrated a negative slope conductance at negative membrane voltages, which is characteristic of NMDA receptor-mediated responses; (iii) kinetics of the individual events composing the dialysis-resistant component of GDPs at negative voltages were very similar to those of AMPA receptor-mediated synaptic currents. Thus, this procedure can be useful to isolate the glutamate receptor-mediated component of neuronal network-driven activities. © 2014 Pleiades Publishing, Ltd.

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Keywords

AMPA receptor, GABA receptor, glutamate, hippocampus, NMDA receptor